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Case Report

Advanced imaging of a histologically confirmed bone infarction of the distal tibia in a Warmblood mare

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Summary

An 8-year-old Warmblood-cross mare presented for investigation of acute onset left hindlimb lameness. Nuclear scintigraphy identified a marked, focal, increase in radiopharmaceutical uptake in the distal aspect of the left tibia. Radiography revealed a large, oval, multi-loculated radiolucent area within the medulla of the distal metaphysis of the left tibia. The mare was treated conservatively for 6 months but showed little improvement in the lameness so the owner elected for euthanasia. Post-mortem computed tomographic imaging revealed a large, oval, hypoattenuating area within the distal tibia, surrounded by a thick, irregular, sclerotic border. The lesion occupied the majority of the medullary cavity but the cortical bone was unaffected. Gross and histopathological examination confirmed a diagnosis of a bone infarction in the medullary cavity of the distal tibia.

Introduction

Bone infarction, also known as osteonecrosis, is the result of a vascular disturbance affecting bone, which leads, eventually, to bone ischaemia and necrosis. Many different factors can induce bone infarction including neoplasia, haematogenous spread of bacterial infection, chronic anaemia, ergot poisoning and trauma (Jubb 2016). In people, risks factors include glucocorticoid therapy, bisphosphonate drugs, HIV infection, alcohol abuse, sickle cell disease, dyslipidaemia and exposure to depressurisation (Dodson 2009; Lafforgue and Trijau 2016). Glucocorticoid-induced osteonecrosis is a well-known entity occurring mainly at the head of the femur and is experimentally induced in several animal species, such as rat, mouse, rabbit, chicken and emu (Xu *et al*. 2018). In contrast to other animals, bone infarction is uncommonly reported in equids and is limited to case reports (Fenger *et al*. 1993; Rantanen *et al*. 1994; Martig *et al*. 2008; Sánchez *et al*. 2010). Affected sites include the long bones such as the femur and tibia. Other cases with similar imaging findings to this case report have also been reported as aneurysmal bone cysts (Ordidge 2001; Bryant *et al*. 2012; David *et al*. 2015). Similar imaging findings were also reported by Stöcker *et al*. (2017) and histopathology of a biopsy from the lesion revealed changes consistent with inflammation, mild fibrosis and bone necrosis; however, no definitive diagnosis was reached. Diagnostic imaging is an invaluable tool to detect osteonecrosis. Radiography is reliable for detecting chronic lesions in people (Munk *et al*. 1989) but advanced imaging techniques such as nuclear scintigraphy and computed tomography may be required for the diagnosis of early lesions and to further characterise chronic lesions.

Here, we describe a case of histologically confirmed osteonecrosis of the distal tibia in a Warmblood mare, including scintigraphic, computed tomographic and radiographic imaging findings.

History

An 8-year-old, bay, Warmblood-cross mare used for general riding presented to the University of Liverpool Equine Hospital for investigation of left hindlimb lameness of 12 weeks duration and recent changes in behaviour including reluctance to flex the left hindlimb. Examination had revealed a mild right forelimb and a moderate left hindlimb lameness. The mare had severely overgrown feet with poor foot balance and remedial farriery had been undertaken prior to referral to address this. The mare was then referred to the hospital for further investigation of the left hind lameness.

Clinical findings

On admission, the mare was bright and clinical parameters were within normal limits. The mare weighed 625 kg with a body condition score of 3/5 (Carroll and Huntington 1988), with good muscle development and symmetry. The mare was reluctant to pick up her left hindlimb. Apart from this, palpation and manipulation of the limbs were unremarkable. At trot in a straight line, the mare showed, subjectively, a moderate (3/5, AAEP) left hindlimb lameness. The forelimbs were normal.

Diagnostic imaging

Nuclear scintigraphic imaging of the axial spine, pelvis, fore and hindlimbs was performed with a gamma camera (Bartec- N-XRD Nucline X-Ring/R Gamma Camera Detector[1]) with bone-phase images obtained three hours after injection

of technetium-99-HDP (10 MBq/kg, intravenously). This identified marked, focal, increased radiopharmaceutical uptake in the distal metaphysis of the left tibia (**Fig 1**). Volumetric ratios showed a 94% increase in radiopharmaceutical uptake in this area compared to the same area in the contralateral limb.

Following a standard 48-h period of isolation to allow for the decay of gamma radiation, lateromedial, dorsoplantar, dorso45°medial-plantarolateral and dorso45°lateral-plantaromedial radiographs of the left tarsus were obtained, including the distal tibia within the margins of collimation (Magnum C-DMS[2]: settings 65 kV and 1.25 mAs), revealing a large, oval, multi-loculated radiolucent area occupying 70% of the medulla of the distal metaphysis of the left tibia (**Fig 2**). The radiolucent area had an irregular trabecular pattern and was bordered by a narrow, radiodense rim.

Diagnosis

Based on the nuclear scintigraphy and radiography findings, a working diagnosis of a multi-loculated cyst-like lesion within the medulla of the distal metaphysis of the left tibia was made. Given the duration of clinical signs and the advanced radiographic changes, this was considered to be a chronic lesion.

Treatment

Treatment options offered included conservative treatment (rest) or surgical debridement and placement of autologous bone graft or bone substitute material as previously reported (David *et al*. 2015; Stöcker *et al*. 2017). The owner elected conservative treatment and the mare was discharged from the hospital. Box rest with daily in-hand grazing was advised for the first 6 weeks followed by small paddock turnout prior to reassessment in 6 months.

Reassessment

The mare represented to the hospital 6 months later for reassessment. On presentation, she was bright and clinical examination was unremarkable. Both hindlimbs were able to be picked up, a significant improvement from initial presentation. However, dynamic examination revealed no improvement in the left hindlimb lameness. Repeat gamma scintigraphy of the hindlimbs revealed increased radiopharmaceutical uptake in the left distal tibia. Volumetric ratios revealed 41% uptake compared to the contralateral limb, markedly less uptake compared to the initial scintigraphic images. Following a standard 48-h period of isolation to allow for the decay of gamma radiation, lateromedial, dorsoplantar, dorso45°medial-plantarolateral and dorso45°lateral-plantaromedial radiographs of the left tarsus were taken, including the distal tibia in the margins of collimation. The lesion in the distal tibia identified previously was still present although the radiodense margin was subjectively thicker and there was a generalised increase in radiopacity of the bone within the lesion. Although there had been improvement, as the mare was still lame the owner declined further treatment (including further rest) and elected for euthanasia.

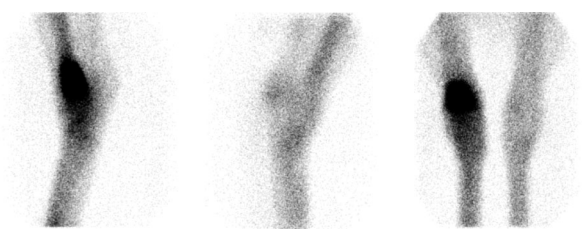

Fig 1: Nuclear scintigraphy revealed marked focal uptake in the distal metaphysis of the left tibia.

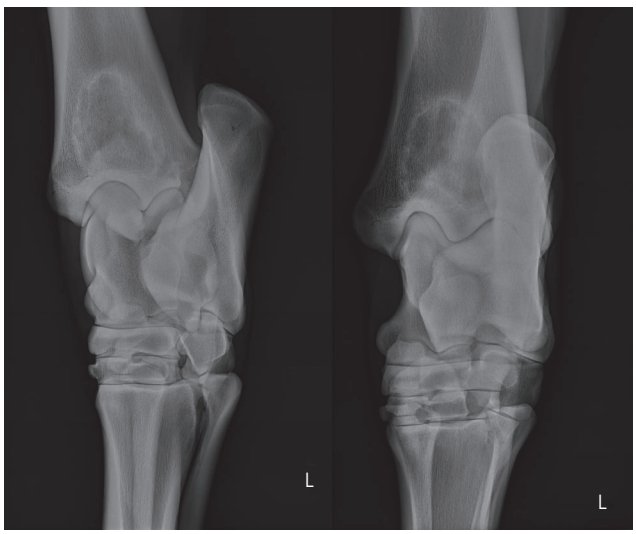

Fig 2: Dorsolateral-plantaromedial oblique (left) and dorsoplantar (right) radiographs of the left hock showing the large, oval, multi-loculated radiolucent area within the medulla of the distal metaphysis of the tibia.

Post-mortem imaging

Following euthanasia, the limb was transected through the proximal tibia and kept in frozen storage for one month. After thawing, computed tomography (CT) of the transected limb was performed (Aquilon-LB 16 slice CT system[3]) with the following technical parameters: 120 kV [peak], 3500 mAs, 1.25 mm contiguous slices. Bone and soft tissue algorithms were used with an image field of view (FOV) of 110 mm diameter and matrix dimensions of 512 × 512. CT revealed a large, oval hypoattenuating area within the distal tibia, surrounded by a thick, irregular, sclerotic border (**Fig 3**). The lesion measured 64 (proximo-distal) × 49 (medial-lateral) × 41 mm (cranio-caudal) and occupied the majority of the medullary cavity of the distal metaphysis and epiphysis of the tibia. The centre of the lesion had an average value of 101 HU while the border had an average value of 88.4 HU. No involvement of cortical bone was evident.

Pathological findings

Macroscopic examination of the thawed and transected bone revealed that the lesion was located in the medulla of the distal metaphysis of the left tibia, with no involvement of the adjacent cortical bone. It was oval-shaped with a creamy coloured centre and a diffusely

Fig 3: Post-mortem 3D MPR-reconstructed CT image of the left tibia. A large oval hypoattenuating area can be seen within the distal tibia, surrounded by a thick, irregular sclerotic border.

orange rim, well delineating the lesion from the surrounding normal bone marrow (**Fig 4a**,**b**). The surface of the tibiotarsal joint was unremarkable with no evidence of articular damage. Popliteal, cranial tibial and caudal tibial arteries and veins were grossly unremarkable with no evidence of thrombosis/vasculitis. Multiple sections of the lesion and surrounding bone were cut with a bandsaw, fixed in 10% neutral buffered formalin and decalcified in RDF Mild Decalcifier[4] for four days and then processed routinely for histological examination. Formalin fixed paraffin embedded (FFPE) sections were cut at 4 μm and stained with haematoxylin and eosin. Histopathology of the core and periphery of the lesion revealed that the centre of the lesion was composed of rarefied trabeculae showing a reduction in size with jagged outlines, empty lacunae and no bone-lining cells, consistent with necrotic bone. The dilated medullary cavities were filled with mature adipocytes, showing occasional hypereosinophilic, irregular granular material consistent with necrotic adipocyte debris (**Fig 4c**,**d**). The outer rim of the lesion was characterised by dense, haphazardly arranged bundles of collagen (myelofibrosis) with proliferating spindle cells, multiple, multinucleated cells (osteoclasts) and macrophages. The macrophages contained fine to large, granular, golden, intracellular pigment consistent with haemosiderin, indicative of chronic haemorrhage. At the periphery, the bone trabeculae were of normal size, with an adequate number of osteocytes and bone-lining cells and a hyperbasophilic line of demarcation was observed between the normal and the necrotic bone (consistent with reversal lines). No signs of osteomyelitis or neoplasia were observed. Small to medium calibre vessels within and around the bone lesion did not show microscopic evidence of thrombosis or vasculitis. The gross and histological morphology was consistent with osteonecrosis (bone infarction).

Discussion

In people, bone infarction is divided into acute and chronic forms. The acute form occurs in subjects showing hyperplastic bone marrow (as in sickle cell disease or Gaucher's disease) and mirrors the clinical signs of an acute osteomyelitis, with severe pain. The chronic form is characterised by a large amount of necrotic and viable medullary adipocytes, without evidence of a haematopoietic component and causes variable pain and clinical symptoms (Lafforgue and Trijau 2016). The reported equine cases (Fenger *et al*. 1993; Rantanen *et al*. 1994; Martig *et al*. 2008; Sánchez *et al*. 2010), including the present one, show a picture overlapping with the chronic form. In this report, a focal lesion in a single bone was affected, although polyostotic lesions are also reported (Fenger *et al*. 1993; Martig *et al*. 2008). According to the few reports published (Fenger *et al*. 1993; Rantanen *et al*. 1994; Martig *et al*. 2008; Sánchez *et al*. 2010), osteonecrosis in horses occurs most commonly in long bones, including the tibia. The blood supply to the distal tibia is provided by branches from the cranial tibial artery, entering the bone through the nutrient foramen (Getty *et al*. 1975). However, no evidence of vascular occlusion in this artery was evident on postmortem or histopathological examination, thus, the underlying cause of the bone infarction in this case remains unclear. Primarily, long bones are affected by bone infarction, most commonly the proximal and distal metaphyses as reported in humans (Lafforgue and Trijau 2016). These findings may indicate that in horses, long bone metaphyses are prone to ischaemic injuries compared to other locations, although further studies are needed to confirm this.

The outcome of bone infarction can be variable, depending on numerous factors including the extension of the lesion and the presence and/or absence of an adequate

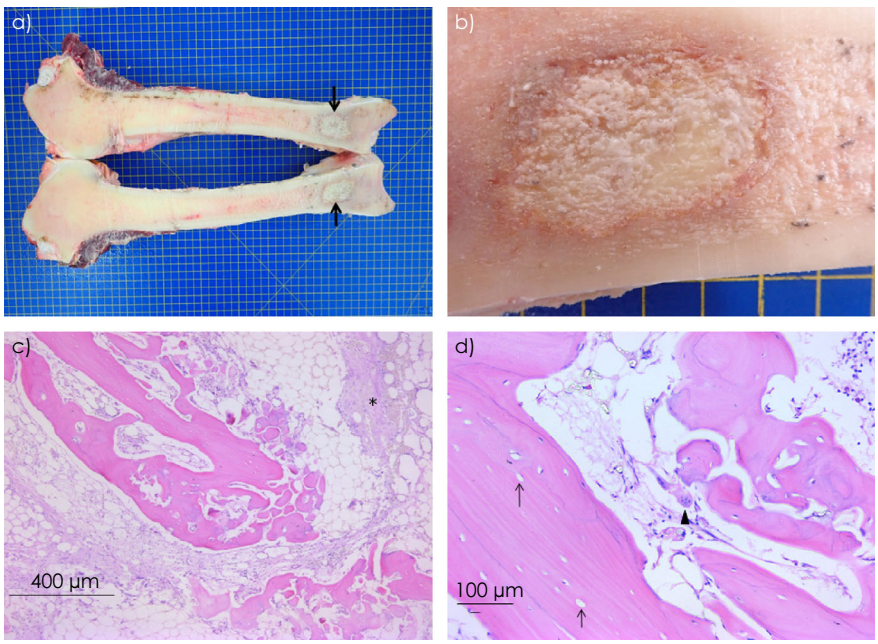

***Fig 4:** a) Longitudinal section of the left tibia, revealing a focal, oval medullary lesion on the distal metaphysis (arrows). b) Close view of the medullary lesion shown in A and lined by an orange to light red rim. Rarefaction of the medullary bone is also noticeable grossly. c) Histological section of the lesion shown in (a) and (b), in which bone trabeculae have a jagged outline, while the bone marrow shows both necrotic and viable adipocytes and myelofibrosis (asterisk). H&E stain, 10×. d) Numerous empty lacunae (arrows) are observed within the bone and without evidence of viable cells (necrotic bone). Isolated osteoclasts (arrowhead) are occasionally observed within the medullar cavities along with evidence of bone resorption (reversal lines). H&E stain, 20×.*

blood supply. Small bone infarctions can heal without intervention but if the lesion is too wide or poorly vascularised, the osteoclasts and osteoblasts are not able to reabsorb and replace the necrotic bone (Jubb 2016). Fenger *et al*. (1993) reported complete healing of bone infarction in a horse, with a return to previous performance and normal radiographic findings. Stöcker *et al*. (2017) reported on successful surgical treatment of a cyst-like lesion in the distal tibial metaphysis of a horse that had very similar imaging findings but different histological findings to the present case. The lesion was debrided and the resultant defect filled with an autologous bone graft and a calcium phosphate bone substitute material. The horse was sound at follow-up examination 6 months postoperatively. Biopsy samples taken at the time of surgery revealed histological evidence of mild inflammation, mild fibrosis and bone necrosis but unlike the present case, was predominantly composed of normal bone and adipocytes. Several histological features previously reported with bone infarction in horses (Fenger *et al*. 1993; Martig *et al*. 2008; Sánchez *et al*. 2010) and evident in the present case were not reported in the case by Stöcker *et al*. (2017). These included evidence of previous haemorrhage (i.e. haemosiderin) and necrosis of adipocytes. As the histopathology was performed on small biopsy samples, it is possible that the samples were not fully representative of the lesion, precluding a definitive diagnosis of bone infarction.

Similar imaging findings have been reported in equine cases of aneurysmal bone cysts (ABC). These have been reported in horses at numerous locations including the mandible and long bones (Steiner and Rendano 1982; Blackwell *et al*. 1985; Lamb and Schelling 1989; Thomas *et al*. 1997; Ordidge 2001; Bryant *et al*. 2012; David *et al*. 2015). ABCs are rare, bone tumour-like lesions, which appear macroscopically as multi-loculated, osseous cysts, typically exuding blood from the cut surface. The cavernous spaces are separated by septi of loosely arranged spindle cells with scattered multinucleate cells and haemosiderin-containing macrophages (Thompson and Dittmer 2016). Due to the absence of any cystic cavity and any blood-filled spaces, the present case is not consistent with an ABC. On radiography, ABCs appear as radiolucent cystic lesions surrounded by a thin layer of cortical bone (Park *et al*. 2016), similar to the radiographic findings in this case.

While some reports of bone infarction in equids (Fenger *et al*. 1993; Martig *et al*. 2008) identified the bone infarct as the cause of the lameness, others (Sánchez *et al*. 2010) described it as an incidental finding. In people, idiopathic osteonecrosis can be an incidental finding. Ideally, diagnostic analgesia would have been performed to further localise the left hindlimb lameness; however, the mare was not amenable to this. In these situations, the clinician is required to rely more on diagnostic imaging. In people, chronic bone infarction is reliably diagnosed using radiography (Munk *et al*. 1989). As with this case, radiodense margins are visible surrounding a lucent area that corresponds to the infarction. However, radiography is considered insensitive for diagnosing acute infarction (Steiner *et al*. 1990). Nuclear scintigraphy is considered more sensitive than radiography for detection of early bone changes associated with bone infarction in people (Assouline-Dayan *et al*. 2002). During the early stages of bone necrosis, both osteoblastic activity and blood flow to the affected area are increased, resulting in increased radiopharmaceutical uptake at the affected site. In people, CT is considered superior to radiography for the diagnosis of

bone infarction, especially of early lesions (Assouline-Dayan *et al*. 2002). In the present case, a CT scan was performed and provided a better appreciation of the size and boundaries of the lesion. Although this was performed post-mortem and so did not change the outcome, it provides a point of reference for future cases of bone infarction in horses. Diagnosis of medullary infarction using magnetic resonance imaging (MRI) has been reported in horses (Sánchez *et al*. 2010). In people, MRI can detect osteonecrosis before abnormalities are visible on radiographs (Assouline-Dayan *et al*. 2002). A double line seen on T2-weighted spin echo sequences, occurring at the boundary of viable and nonviable tissue is characteristic of bone infarction in people (Saini and Saifuddin 2004) and has been reported in horses (Sánchez *et al*. 2010). However, nuclear scintigraphy is considered to be more sensitive than MRI for the detection of early lesions in people (Koo *et al*. 1994).

Conclusion

This report outlines the clinical and imaging findings of a confirmed case of bone infarction in the distal tibial metaphysis of an adult horse. By presenting the findings of multiple imaging modalities, along with gross pathology and histopathology findings, this report confirms that bone infarction can be established as a clinical diagnosis using nuclear scintigraphic, radiographic and computed tomographic findings. However, histopathology remains essential to make a definitive diagnosis and to differentiate between bone infarction and aneurysmal bone cysts.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

The owner of the horse in this case report provided ethical consent for use of case details.

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None.

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Authorship

C. Robinson was responsible for initial investigation and referral of the case. M. Hann, M.C Martins and P. Milner were involved in the work-up, diagnosis and treatment of the case; G. Rocchigiani and R. Verin with the gross pathology and histopathology. All authors contributed to and gave their final approval of the manuscript.

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[4]Cellpath, Newtown, Wales, UK.

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